3120466

JP No.~~3126466~~

CLAIMS

(57) [Claim(s)]

[Claim 1]

It is the amplifying device of the deoxyribonucleic acid characterized by providing the equipment for sending in said reaction mixture in the capillary which contained the reaction mixture containing the deoxyribonucleic acid which should be amplified, and said capillary, and the equipment for controlling said capillary part to predetermined temperature, and for said reaction mixture in said capillary being closed by the gas for sending in said reaction mixture by the position in said capillary, and being transported to a position.

[Claim 2]

The equipment which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, The 1st, 2nd, and 3rd equipment which holds the thermal denaturation temperature in the PCR method, annealing temperature, and polymerization temperature, respectively is provided. The amplifying device of the deoxyribonucleic acid characterized by performing the PCR method which makes a deoxyribonucleic acid amplify when only the count of predetermined repeats changing the temperature of said reaction mixture in said capillary to said thermal denaturation temperature, said annealing temperature, and said polymerization temperature one by one.

[Claim 3]

The equipment which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, By providing the 1st, 2nd, and 3rd equipment which holds the thermal denaturation temperature in the PCR method, annealing temperature, and polymerization temperature, respectively, and supplying or removing air or other gas from one side of said capillary Said reaction mixture in said capillary one by one Said thermal denaturation temperature, said annealing temperature, And when only the count of predetermined repeats, making it move to the location where said polymerization temperature is held, and changing the temperature of said reaction mixture in said capillary to said thermal denaturation temperature, said annealing temperature, and said polymerization temperature one by one The

amplifying device of the deoxyribonucleic acid characterized by performing the PCR method which makes a deoxyribonucleic acid amplify.

[Claim 4]

The equipment which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, The support means which supports said capillary, and the migration means to which said capillary is moved, The 1st, 2nd, and 3rd equipment which holds the thermal denaturation temperature in the PCR method, annealing temperature, and polymerization temperature, respectively is provided. Said capillary is moved to the 1st, 2nd, and 3rd equipment one by one with said migration means. The amplifying device of the deoxyribonucleic acid characterized by performing the PCR method which makes a deoxyribonucleic acid amplify when only the count of predetermined repeats changing the temperature of said reaction mixture in said capillary to said thermal denaturation temperature, said annealing temperature, and said polymerization temperature one by one.

[Claim 5]

The equipment which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified by gas across ends, and controls migration of said reaction mixture in said capillary, It is the amplifying device of the deoxyribonucleic acid characterized by providing the equipment which holds the temperature of said reaction mixture in said capillary to predetermined temperature, and for said reaction mixture in said capillary being closed by said gas by the position in said capillary, and being transported to a position.

[Claim 6]

The equipment which puts in the reaction mixture containing the deoxyribonucleic acid which should be amplified into the capillary rolled two or more times spirally by ****** which does not come by air or other gas across ends, Thermal denaturation temperature [in / to the hoop direction of said capillary rolled two or more times spirally / the PCR method], 1st, 2nd, and 3rd means to hold annealing temperature and polymerization temperature, respectively are arranged in predetermined order. The amplifying device of the deoxyribonucleic acid characterized by performing the PCR method which makes a deoxyribonucleic acid amplify when only the count of predetermined repeats changing the temperature of said reaction mixture in said capillary to said thermal denaturation temperature, said annealing temperature, and said polymerization temperature one by one.

[Claim 7]
(1) The process which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, (2) The process which holds the temperature of said reaction mixture in said capillary to the thermal denaturation temperature in the PCR method, (3) The process which holds the temperature of said reaction mixture in said capillary to the annealing temperature in the PCR method, (4) The magnification approach of the deoxyribonucleic acid characterized by performing the PCR method which it has [PCR method] the process which holds the temperature of said reaction mixture in said capillary to the polymerization temperature in the PCR method, and only the count of predetermined repeats [PCR method] said process (4) one by one from said process (2), and makes a deoxyribonucleic acid amplify.
[Claim 8]
(1) The process which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, (2) by supplying or removing air or other gas from one side of said capillary the process which is made to move said reaction mixture in said capillary to the equipment with which the thermal denaturation temperature in the PCR method is held, and holds said reaction mixture to said thermal denaturation temperature, and (3) -- by supplying or removing air or other gas from one side of said capillary The process which is made to move said reaction mixture in said capillary to the equipment with which the annealing temperature in the PCR method is held, and holds said reaction mixture to said annealing temperature, (4) by supplying or removing air or other gas from one side of said capillary Said reaction mixture in said capillary is moved to the equipment with which the polymerization temperature in the PCR method is held. The magnification approach of the deoxyribonucleic acid characterized by performing the PCR method which it has [PCR method] the process which holds said reaction mixture to said polymerization temperature, and only the count of predetermined repeats [PCR method] said process (4) one by one from said process (2), and makes a deoxyribonucleic acid amplify.
[Claim 9]
(1) The process which puts in said reaction mixture into a capillary by ****** which does not come the reaction mixture containing the deoxyribonucleic acid which should be amplified across ends by air or other gas, (2) The process which is made to move said capillary to the equipment with which thermal denaturation temperature [in / for said reaction mixture in said capillary / the PCR method] is held, and holds said reaction

mixture to said thermal denaturation temperature, (3) The process which is made to move said capillary to the equipment with which annealing temperature [in / for said reaction mixture in said capillary / the PCR method] is held, and holds said reaction mixture to said annealing temperature, (4) Said capillary is moved to the equipment with which polymerization temperature [in / for said reaction mixture in said capillary / the PCR method] is held. The magnification approach of the deoxyribonucleic acid characterized by performing the PCR method which it has [PCR method] the process which holds said reaction mixture to said polymerization temperature, and only the count of predetermined repeats [PCR method] said process (4) one by one from said process (2), and makes a deoxyribonucleic acid amplify.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application]

This invention relates to the amplifying device and the magnification approach of a deoxyribonucleic acid which used capillaries, such as a capillary tube, especially as a container of reaction mixture about magnification of the deoxyribonucleic acid using the PCR method.

[0002]

[Description of the Prior Art]

JP,62-240862,A is indicated as a conventional technique of the amplifying device of the deoxyribonucleic acid using the PCR method. moreover, the approach of using a capillary tube as a container of reaction mixture -- Analytical Biochemistry and 186 (1990) -- it is indicated by 331 pages (Analytical Biochemistry 186(1990) pp 328-331) from the 328th page.

[0003]

[Problem(s) to be Solved by the Invention]

However, a plastic envelope with the lid of throwing away as a container of reaction mixture in the conventional technique which above-mentioned JP,62-240862,A is indicating The activity of (a 0.5ml micro HYUJI tube [for example,]) is assumed. About 0.1ml It puts into a plastic envelope with the lid of throwing away which described the reaction mixture of extent above. Furthermore, the straight mineral oil for preventing evaporation of the moisture in reaction mixture is superimposed on the upper layer of reaction mixture. Although usually carry out a repetition temperature change to order

with the thermal denaturation temperature of about 95 degrees C, an annealing temperature [of about 55 degrees C], and a polymerization temperature of about 70 degrees C dozens times, the PCR method is made to perform and the deoxyribonucleic acid is amplified There is a fault for which the clearance process of straight mineral oil is needed after magnification reaction termination. Moreover, the heat capacity of the plastic envelope with a disposable lid is also large, and since heat transfer to reaction mixture is also bad, it has the fault to which the time amount which performs the PCR method becomes long. The conventional technique which 331 pages is indicating from the 328th page uses a capillary tube as a container of reaction mixture. on the other hand -- above-mentioned Analytical Biochemistry and 186 (1990) -- Although time amount which makes the heat capacity of a container small, and improves heat transfer to reaction mixture, and performs the PCR method is shortened while preventing evaporation of the moisture in reaction mixture by closing the ends of a capillary tube with combustion gas, after putting in reaction mixture into a capillary tube There is a fault for which the actuation which takes out reaction mixture from the closed capillary tube after the actuation which closes the ends of a capillary tube with combustion gas, and magnification reaction termination is needed. Moreover, since processes, such as an extract of the object DNA and a sequence reaction, are before and after that, although magnification of DNA using the PCR method is one processes, such as a gene analysis and gene diagnosis, and continuity with the process of these order is important for it, with the conventional technique, the consideration to continuity with the process of order is not made.

[0004]

While this invention is what was made in view of the fault of said conventional technique, making the clearance process of straight mineral oil unnecessary, shortening time amount which performs the PCR method and making unnecessary the actuation which takes out reaction mixture from the capillary tube which closes the ends of a capillary tube with combustion gas, and which was operated and closed, it is in offering the amplifying device and the magnification approach of a deoxyribonucleic acid which were taken into consideration also to continuity with the process before and behind the magnification process of DNA using the PCR method.

[0005]

[Means for Solving the Problem] The above-mentioned object is attained by changing the reaction mixture which put in reaction mixture into capillaries, such as a capillary tube, and described it above into the condition of having inserted ends by air or other gas, paying attention to the ability preventing substantially because the moisture

evaporation from the gas-liquid interface of reaction mixture closes the reaction mixture in a capillary by gas, and making the PCR method perform.

[0006]

[Function] In order to perform the PCR method, the repetition temperature change of the reaction mixture is usually carried out dozens times, in that case, if moisture evaporation occurs in the order of about 95-degree thermal denaturation temperature of C about, about C annealing temperature of about 55 degrees, and about C about 70-degree polymerization temperature, the presentation of reaction mixture changes to it and the PCR method cannot perform it in it as the object. However, since the moisture evaporation from reaction mixture occurs by the interface of reaction mixture and gas, if area of said interface is made sufficiently small, when moisture evaporation can be made sufficiently small and it will perform the PCR method, it can be carried out to presentation change of the reaction mixture of extent which does not produce trouble. Furthermore, since migration of reaction mixture is controllable by said gas, the amplifying device of a deoxyribonucleic acid taken into consideration also to continuity with the process before and behind the DNA magnification process using the PCR method and the magnification approach can be realized easily.

[0007]

[Example] Drawing 1 is the block diagram showing one example, and, for a reaction mixture feed hopper and 3, as for a reaction mixture exhaust port and 6, a gas air supplying opening and 4 are [the capillary tube whose bore of 1 is about 1mm and 2 / a cross valve and 7] stop valves. 8 is capillary tube support, 9 is a capillary tube migration device, and this controls the location of the capillary tube support 8. Although omitted, if migration is smoothly controllable in short, the configuration of arbitration can take both detail. 21a, 22a, and 23a are containers, respectively. 21, 22, and 23 are heat carriers and are in Containers 21a, 22a, and 23a, respectively. [close] Each heat carrier is maintained by the denaturation temperature of reaction mixture, annealing temperature, and polymerization temperature. 11 is reaction mixture and is in the condition currently closed by gas in the capillary tube. Hereafter, actuation is explained according to drawing 1 . Contamination of reaction mixture is prevented by passing a penetrant remover for the part which reaction mixture, such as inside of a capillary tube 1 and the cross valve 6 stop valve 7, passes beforehand instead of reaction mixture, with the capillary tube support 8, after putting in a capillary tube 1 into a heat carrier 21, a cross valve 6 and a stop valve 7 are operated, and the reaction mixture feed hopper 2, a capillary tube 1 and a capillary tube 1, and the reaction mixture exhaust port 4 are made to open for free passage according to the capillary tube migration device 9,

respectively. After putting in reaction mixture 11 into a capillary tube 1 from the reaction mixture feed hopper 2, operate a cross valve 6 and the gas air supplying opening 3 and a capillary tube 1 are made to open for free passage, and gas is supplied from the gas air supplying opening 3 so that reaction mixture 11 may come to the position in a capillary tube 1. If predetermined after [time amount of] reaction mixture 11 becomes thermal denaturation temperature, the capillary tube migration device 9 connected with the capillary tube support 8 will be operated, and a capillary tube 1 will be put in into a heat carrier 22. If predetermined after [time amount of] reaction mixture 11 becomes annealing temperature, the capillary tube migration device 9 connected with the capillary tube support 8 will be operated, and a capillary tube 1 will be put in into a heat carrier 23. If predetermined after [time amount of] reaction mixture 11 becomes polymerization temperature, the capillary tube migration device 9 connected with the capillary tube support 8 will be operated, and a capillary tube 1 will be put in into a heat carrier 21. Hereafter, migration of a capillary tube 1 is repeated in the same sequence, after only a predetermined count carries out the temperature change of the reaction mixture to the order of thermal denaturation temperature, annealing temperature, and polymerization temperature and enforces the PCR method, gas is supplied from the gas air supplying opening 3, and reaction mixture 11 is discharged from the reaction mixture exhaust port 4. Operate a cross valve 6 and the gas air supplying opening 3 and a capillary tube 1 are made to open for free passage, and in case gas is supplied from the gas air supplying opening 3 so that reaction mixture 11 may come to the position in a capillary tube 1, a stop valve 7 is operated and you may make it internal pressure suitable in a capillary tube 1 applied in the above actuation. If it does in this way, even if gas, such as air of a minute amount, will mix into reaction mixture 11 temporarily, it is effective in the ability to prevent fragmentation of the reaction mixture accompanying expansion of the gas by the temperature change of reaction mixture.

[0008]

Now, using the capillary tube with a bore [of 1mm], and an outer diameter of 2mm made from plastics as an example, if the temperature change of the reaction mixture when putting in a capillary tube into the 55-degree C warm water of annealing temperature from 95-degree C thermal denaturation temperature is searched for by numerical calculation and shown as time amount change of the mean temperature of reaction mixture where reaction mixture is put into a capillary tube, it will become like drawing 2 . This drawing shows that the temperature of reaction mixture turns into annealing temperature of 95-degree C thermal denaturation temperature to about 55

degrees C in about 15 seconds. Namely, the time amount which a series of temperature changes of thermal denaturation temperature, annealing temperature, and polymerization temperature take even if it puts in the transit time of a capillary tube is about 1 minute, and even if it repeats it about 30 times, it can enforce the PCR method in about 30 minutes. Although a count result is not shown, if the diameter of inside and outside is set to one half, respectively, the PCR method can be enforced in about 15 minutes.

[0009]

Drawing 3 is the block diagram showing other examples, for a gas air supply and exhaust opening and 4, a reaction mixture exhaust port, and 61 and 62 are [a reaction mixture feed hopper, and 3 and 5 / a capillary tube and 2 / a heat carrier, and 21a, 22a and 23a of a cross valve and 21, 22 and 23] containers, and the heat carrier which is in this is maintained for 1 by thermal denaturation temperature, annealing temperature, and polymerization temperature, respectively. 11 is reaction mixture. The example of drawing 3 is carried out to comparing with it of drawing 1 , replacing with migration of a capillary tube 1, and moving reaction mixture 11 the very thing. Hereafter, actuation is explained according to drawing 3 . After preventing contamination of reaction mixture by passing a penetrant remover for the part which reaction mixture, such as inside of a capillary tube 1 and cross valves 61 and 62, passes beforehand instead of reaction mixture, cross valves 61 and 62 are operated and the reaction mixture feed hopper 2, a capillary tube 1 and a capillary tube 1, and the gas air supply and exhaust opening 5 are made to open for free passage, respectively. After putting in reaction mixture 11 into a capillary tube 1 from the reaction mixture feed hopper 2, operate a cross valve 61 and the gas air supply and exhaust opening 3 and a capillary tube 1 are made to open for free passage, and gas is supplied from the gas air supply and exhaust opening 3 so that it may come to the position in the capillary tube 1 with which reaction mixture 11 is flooded with the heat carrier 21. If predetermined after [time amount of] reaction mixture 11 becomes thermal denaturation temperature, gas will be supplied from the gas air supply and exhaust opening 3 so that it may come to the position in the capillary tube 1 with which reaction mixture 11 is flooded with the heat carrier 22. If predetermined after [time amount of] reaction mixture 11 becomes annealing temperature, gas will be supplied from the gas air supply and exhaust opening 3 so that it may come to the position in the capillary tube 1 with which reaction mixture 11 is flooded with the heat carrier 23. If predetermined after [time amount of] reaction mixture 11 becomes polymerization temperature, gas will be supplied from the gas air supply and exhaust opening 5 so that it may come to the position in the capillary tube 1

with which reaction mixture 11 is flooded with the heat carrier 21. Hereafter, migration within the capillary tube 1 of reaction mixture 11 is repeated, after only a predetermined count carries out the temperature change of the reaction mixture to the order of thermal denaturation temperature, annealing temperature, and polymerization temperature and enforces the PCR method, operate a cross valve 62 and a capillary tube 1 and the reaction mixture exhaust port 4 are made to open for free passage, gas is supplied from the gas air supply and exhaust opening 3, and reaction mixture 11 is discharged from the reaction mixture exhaust port 4. Of course, when the backward feed for a repetition of reaction mixture, it cannot be overemphasized that it is made for there to be no effect by this backward feed as controlling to return for a short time. Moreover, when supplying gas and repeating migration within the capillary tube 1 of reaction mixture 11 from the gas air supply and exhaust openings 3 and 5, you may make it internal pressure suitable in a capillary tube 1 applied. This effectiveness is the same as the 1st example. When the capillary tube of the same dimension as the 1st example is used by this example, since the capillary tube is already the target temperature, it is guessed easily that the time amount taken for the temperature of reaction mixture to turn into the target temperature is shorter than the 1st example. Moreover, in this invention, since migration of reaction mixture is performed by the air supply and exhaust of gas, there is almost no moving part, it is cheap and there is effectiveness which can be used as reliable equipment.

[0010]

Drawing 4 is the block diagram showing the example of further others, and 1 is spirally rolled with the capillary tube. For 2, as for a gas air supplying opening and 4, a reaction mixture feed hopper and 3 are [a reaction mixture exhaust port and 6] cross valves.

[0011]

31, 32, and 33 are maintained by thermal denaturation temperature, annealing temperature, and polymerization temperature with a heat block, respectively, and the capillary tube 1 is spirally rolled, where this is contacted enough thermally. 11 is reaction mixture. The spiral number of turns of a capillary tube 1 are carried out more than the required count of the PCR method which repeats a temperature change in order of thermal denaturation temperature, annealing temperature, and polymerization temperature. Hereafter, actuation is explained according to drawing 4 . Contamination of reaction mixture is prevented, a cross valve 6 is operated, and the reaction mixture feed hopper 2 and a capillary tube 1 are made to open for free passage by passing a penetrant remover for the part which reaction mixture, such as inside of a capillary

tube 1 and a cross valve 6, passes beforehand instead of reaction mixture. After putting in reaction mixture 11 into a capillary tube 1 from the reaction mixture feed hopper 2, operate a cross valve 6 and the gas air supplying opening 3 and a capillary tube 1 are made to open for free passage, and gas is supplied from the gas air supplying opening 3 so that reaction mixture 11 may come to the position of the heat block 31. If predetermined after [time amount of] reaction mixture 11 becomes thermal denaturation temperature, gas will be supplied from the gas air supplying opening 3 so that reaction mixture 11 may come to the position of the heat block 32. If predetermined after [time amount of] reaction mixture 11 becomes annealing temperature, gas will be supplied from the gas air supplying opening 3 so that reaction mixture 11 may come to the position of the heat block 33. Hereafter, the same actuation is repeated, after only a predetermined count carries out the temperature change of the reaction mixture to the order of thermal denaturation temperature, annealing temperature, and polymerization temperature and enforces the PCR method, it is made quicker than the rate which described the speed of supply of gas above from the gas air supplying opening 3, and continuation supply is carried out and reaction mixture 11 is discharged from the reaction mixture exhaust port 4. If the gas supply rate from an air supplying opening 3 is controlled suitably to become the target temperature in the above mentioned actuation while reaction mixture 11 moves, gas can be continuously supplied for the PCR method as **** R>. In addition, flow-resistance components, such as drawing, are prepared in the suitable location before and behind the reaction mixture exhaust port 4, and you may make it internal pressure suitable in a capillary tube 1 applied. This effectiveness is the same as the 1st example. When the capillary tube of the same dimension as the 1st example is used by this example, since the capillary tube is already the target temperature, it is guessed easily that the time amount taken for the temperature of reaction mixture to turn into the target temperature is shorter than the 1st example. Moreover, in this example, since the PCR method is performed when reaction mixture moves to an one direction, while charging control of gas is easy, since there is no need, backward feed [the location which is in an unsuitable temperature condition about reaction mixture], control is possible for high degree of accuracy. Furthermore, there is almost no moving part, it is cheap and there is effectiveness which can be used as reliable equipment.

[0012]

It is as follows if it estimates about the evaporation in the interface of reaction mixture.

[0013]

the area of the interface when putting reaction mixture into a plastic envelope with the

aforementioned lid -- about 35 -- mm2 -- on the other hand -- a capillary tube with a bore of 1mm -- in all [ends] -- since it is 2 about 1.6mm, 1/20 or less is the area of an interface. If the capillary tube of a still thinner bore uses it if needed, it is also easy to make area of an interface still smaller. If area of an interface is made small, it will be proved also with the following data that moisture evaporation can be made sufficiently small. Moisture evaporation is 0.1%, even if it changes into the condition of having inserted ends with air the reaction mixture which put in and described reaction mixture above in said capillary tube and puts it into the 95-degree C thermostatic chamber for 10 minutes using the capillary tube with a bore of 1mm made from plastics. It was extent. If temperature is low, it is obvious that moisture evaporation becomes still smaller. Namely, if the reaction mixture which put in and described reaction mixture above in said capillary tube is changed into the condition of having inserted ends by air or other gas, using a capillary tube with a bore of about 1mm or less as a container of reaction mixture It is not necessary to use the straight mineral oil currently used with the conventional technique. Again Analytical Biochemistry and 186 (1990) -- as shown to 331 pages (Analytical Biochemistry 186(1990) pp 328-331) from the 328th page Even if it does not close capillary tube order, when performing the PCR method, the target PCR method can be performed by presentation change of the reaction mixture of extent which does not produce trouble.

[0014]

[Effect of the Invention]

The clearance process of straight mineral oil which is the fault of said conventional technique according to this invention as explained above Or the actuation which takes out reaction mixture from the capillary tube which closes the ends of a capillary tube with combustion gas, and which was operated and closed is unnecessary. And since it the PCR method which can be processed in a short time is not only realizable, but is devised so that supply of reaction mixture and blowdown can follow the head end process of the PCR method, and a tail end process, respectively The amplifying device of a deoxyribonucleic acid with continuity with the process before and behind the magnification process of DNA using the PCR method is realizable.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is the block diagram showing one example of this invention.

[Drawing 2] It is drawing showing an example of the average temperature change of

reaction mixture.

[Drawing 3] It is the block diagram showing other examples of this invention.

[Drawing 4] It is the block diagram showing other examples of this invention.

[Description of Notations]

1 -- A capillary tube,

2 -- A reaction mixture feed hopper,

3 -- A gas air supplying opening

4-- A reaction mixture exhaust port,

5 / -- A gas air supply and exhaust opening,

6 61, 62 / -- A cross valve

7 are a stop valve,

21, 22 and 23. / -- It is a heat carrier,

31, 32 and 33. / -- It heat-blocks.]

図 1

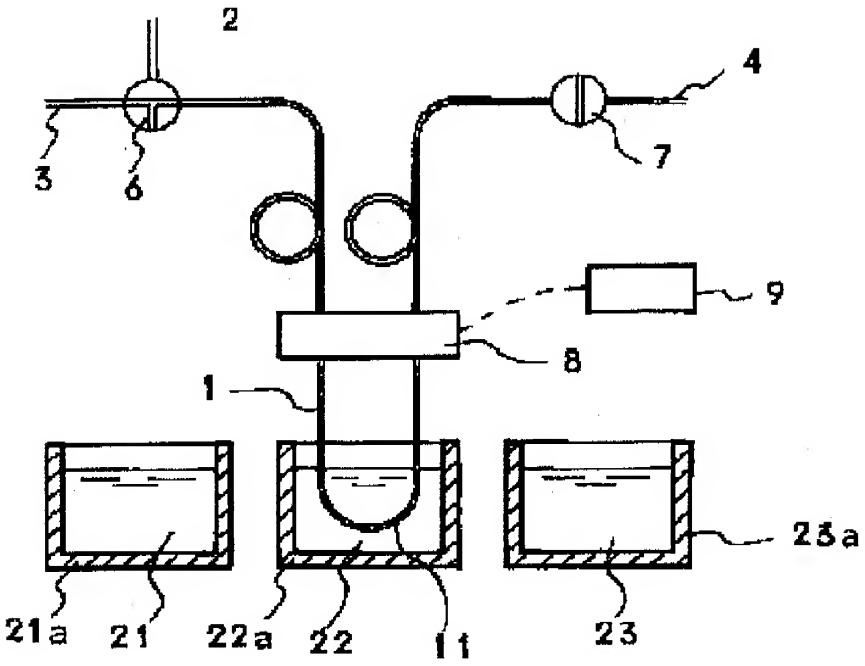


図 2

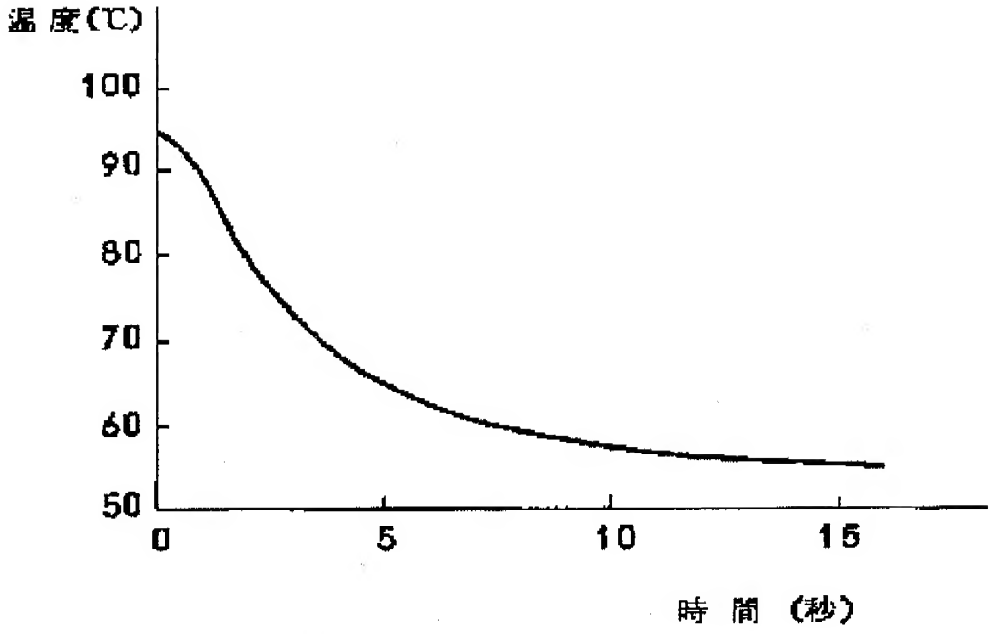


図 3

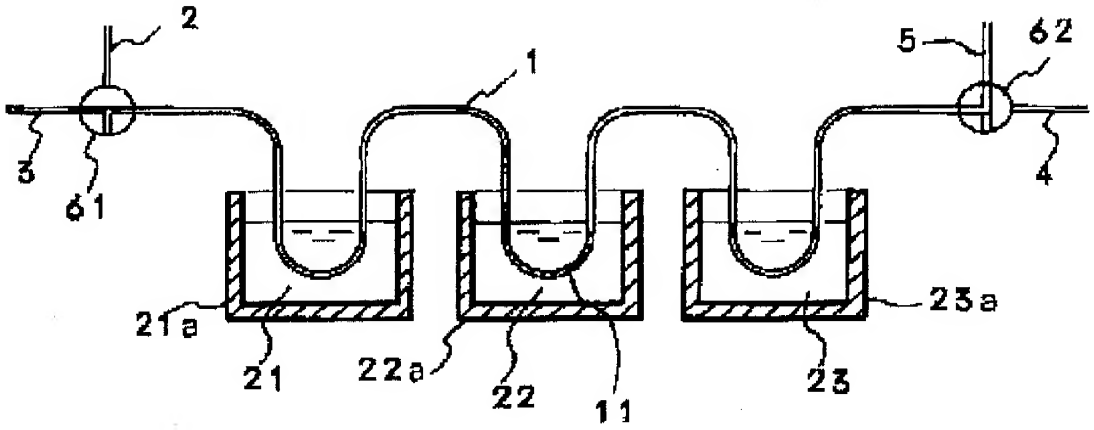
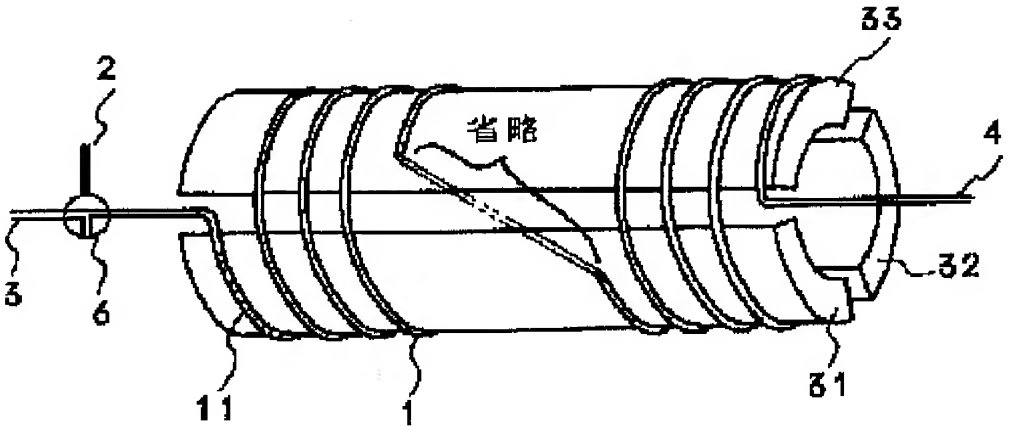


図 4



(19)日本国特許庁（ＪＰ）　(12)特　許　公　報（Ｂ２）　(11)特許番号
特許第3120466号
(P3120466)

(45)発行日　平成12年12月25日(2000.12.25)　(24)登録日　平成12年10月20日(2000.10.20)

(51)Int.Cl.[7]	識別記号	ＦＩ	
Ｃ１２Ｍ　1/00		Ｃ１２Ｍ　1/00	Ａ
Ｃ１２Ｑ　1/68		Ｃ１２Ｑ　1/68	Ａ

請求項の数９(全　６　頁)

(21)出願番号　特願平3－95498

(22)出願日　平成３年４月25日(1991.4.25)

(65)公開番号　特開平4－325080

(43)公開日　平成４年11月13日(1992.11.13)

審査請求日　平成10年３月24日(1998.3.24)

(73)特許権者　000005108
株式会社日立製作所
東京都千代田区神田駿河台四丁目６番地

(72)発明者　臼井　三平
埼玉県比企郡鳩山町赤沼2520番地　株式会社　日立製作所　基礎研究所内

(72)発明者　藤田　雅彦
埼玉県比企郡鳩山町赤沼2520番地　株式会社　日立製作所　基礎研究所内

(74)代理人　100075096
弁理士　作田　康夫

審査官　鈴木　恵理子

最終頁に続く

(54)【発明の名称】　デオキシリボ核酸の増幅装置及び増幅方法

1

(57)【特許請求の範囲】

【請求項１】増幅すべきデオキシリボ核酸を含む反応液を収納した細管と、前記細管内に前記反応液を送りこむための装置と、前記細管部分を所定の温度に制御するための装置とを具備し、前記細管内の前記反応液は、前記反応液を送りこむためのガスで前記細管内の所定の位置に封止され且つ所定の位置に移送されることを特徴とするデオキシリボ核酸の増幅装置。

【請求項２】増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる装置と、ＰＣＲ法に於ける熱変性温度、アニーリング温度、及び重合温度をそれぞれ保持する第１、第２、及び第３の装置とを具備し、前記細管の中の前記反応液の温度を、順次、前記熱変性温度、前記アニーリング温度、及び前記重合温度に変化させることを所定回数だけ繰返すことにより、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅装置。

2

【請求項３】増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる装置と、ＰＣＲ法に於ける熱変性温度、アニーリング温度、及び重合温度をそれぞれ保持する第１、第２、及び第３の装置とを具備し、前記細管の一方から空気もしくは他のガスを供給もしくは除去することにより、前記細管内の前記反応液を、順次、前記熱変性温度、前記アニーリング温度、及び前記重合温度が保持される位置に移動させて、前記細管の中の前記反応液の温度を、順次、前記熱変性温度、前記アニーリング温度、前記重合温度に変化させることを所定回数だけ繰返すことにより、デオキシリボ核酸を増幅させる

ＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅装置。

【請求項４】増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる装置と、前記細管を支持する支持手段と、前記細管を移動させる移動手段と、ＰＣＲ法に於ける熱変性温度、アニーリング温度、及び重合温度をそれぞれ保持する第１、第２、及び第３の装置とを具備し、前記移動手段により前記細管を、順次、第１、第２、及び第３の装置に移動させて、前記細管の中の前記反応液の温度を、順次、前記熱変性温度、前記アニーリング温度、及び前記重合温度に変化させることを所定回数だけ繰返すことにより、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅装置。

【請求項５】増幅すべきデオキシリボ核酸を含む反応液をガスで両端を挟みこんだ状態で前記反応液を細管の中に入れ、前記細管内の前記反応液の移送を制御する装置と、前記細管内の前記反応液の温度を所定の温度に保持する装置とを具備し、前記細管内の前記反応液は前記ガスで前記細管内の所定の位置に封止され且つ所定の位置に移送されることを特徴とするデオキシリボ核酸の増幅装置。

【請求項６】螺旋状に複数回巻かれた細管の中に、増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で入れる装置と、螺旋状に複数回巻かれた前記細管の周方向に、ＰＣＲ法に於ける熱変性温度、アニーリング温度、及び重合温度をそれぞれ保持する第１、第２、及び第３の手段とが所定の順に配置され、前記細管の中の前記反応液の温度を、順次、前記熱変性温度、前記アニーリング温度、及び前記重合温度に変化させることを所定回数だけ繰返すことにより、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅装置。

【請求項７】（１）増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる工程と、（２）前記細管の中の前記反応液の温度をＰＣＲ法に於ける熱変性温度に保持する工程と、（３）前記細管の中の前記反応液の温度をＰＣＲ法に於けるアニーリング温度に保持する工程と、（４）前記細管の中の前記反応液の温度をＰＣＲ法に於ける重合温度に保持する工程とを有し、前記工程（２）から前記工程（４）を、順次、所定回数だけ繰返し、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅方法。

【請求項８】（１）増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる工程と、（２）前記細管の一方から空気もしくは他のガスを供給もしくは除去することにより、前記細管内の前記反応液をＰＣＲ法に

於ける熱変性温度が保持される装置に移動させて、前記反応液を前記熱変性温度に保持する工程と、（３）前記細管の一方から空気もしくは他のガスを供給もしくは除去することにより、前記細管内の前記反応液をＰＣＲ法に於けるアニーリング温度が保持される装置に移動させて、前記反応液を前記アニーリング温度に保持する工程と、（４）前記細管の一方から空気もしくは他のガスを供給もしくは除去することにより、前記細管内の前記反応液をＰＣＲ法に於ける重合温度が保持される装置に移動させて、前記反応液を前記重合温度に保持する工程とを有し、前記工程（２）から前記工程（４）を、順次、所定回数だけ繰返し、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅方法。

【請求項９】（１）増幅すべきデオキシリボ核酸を含む反応液を空気もしくは他のガスで両端を挟みこんだ状態で前記反応液を細管の中に入れる工程と、（２）前記細管内の前記反応液をＰＣＲ法に於ける熱変性温度が保持される装置に前記細管を移動させて、前記反応液を前記熱変性温度に保持する工程と、（３）前記細管内の前記反応液をＰＣＲ法に於けるアニーリング温度が保持される装置に前記細管を移動させて、前記反応液を前記アニーリング温度に保持する工程と、（４）前記細管内の前記反応液をＰＣＲ法に於ける重合温度が保持される装置に前記細管を移動させて、前記反応液を前記重合温度に保持する工程とを有し、前記工程（２）から前記工程（４）を、順次、所定回数だけ繰返し、デオキシリボ核酸を増幅させるＰＣＲ法を実行することを特徴とするデオキシリボ核酸の増幅方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明はＰＣＲ法を使ったデオキシリボ核酸の増幅に関し、特に反応液の容器として毛細管等の細管を使用したデオキシリボ核酸の増幅装置及び増幅方法に関する。

【０００２】

【従来の技術】ＰＣＲ法を使ったデオキシリボ核酸の増幅装置の従来技術として、特開昭62－240862号が開示されている。また、反応液の容器として毛細管を使用する方法がアナリティカル・バイオケミストリ，１８６（１９９０）第３２８頁から３３１頁（Analytical Biochemistry　１８６(１９９０）ｐｐ３２８－３３１）に記載されている。

【０００３】

【発明が解決しようとする課題】しかしながら、上記特開昭62－240862号が開示している従来技術は反応液の容器として使い捨ての蓋付のプラスチック容器（例えば、０．５ｍｌ のマイクロヒュージチューブ）の使用を想定し、およそ０．１ｍｌ 程度の反応液を前記した使い捨ての蓋付のプラスチック容器に入れ、さらに反応液の上層

に反応液中の水分の蒸発を防止するための鉱物油を重畳し、およそ９５℃程度の熱変性温度、およそ５５℃程度のアニーリング温度、およそ７０℃程度の重合温度の順に、通常数十回繰返し温度変化させてＰＣＲ法を行わせ、デオキシリボ核酸の増幅を行っているが、増幅反応終了後、鉱物油の除去工程が必要となる欠点がある。また、使い捨ての蓋付のプラスチック容器は、熱容量も大きく、反応液への熱伝達も悪いため、ＰＣＲ法を行う時間が長くなる欠点がある。一方、上記アナリティカル・バイオケミストリ，１８６（１９９０）第３２８頁から３３１頁が開示している従来技術は反応液の容器として毛細管を使用し、反応液を毛細管の中に入れた後、毛細管の両端を燃焼ガスで封止することにより、反応液中の水分の蒸発を防止するとともに、容器の熱容量を小さくし、かつ反応液への熱伝達を良くしてPCR法を行う時間を短くしているが、毛細管の両端を燃焼ガスで封止する操作、増幅反応終了後、封止した毛細管から反応液を取り出す操作が必要となる欠点がある。また、ＰＣＲ法を用いたＤＮＡの増幅は、遺伝子解析や遺伝子診断等の一工程であり、その前後には目的ＤＮＡの抽出，シーケンス反応等の工程があるので、それら前後の工程との継続性が重要であるにもかかわらず、従来技術では前後の工程との継続性に対する考慮がなされていない。

【０００４】本発明は前記従来技術の欠点に鑑みてなしたもので、鉱物油の除去工程を不要とし、ＰＣＲ法を行う時間を短くし、毛細管の両端を燃焼ガスで封止する操作、ならびに封止した毛細管から反応液を取り出す操作を不要とするとともに、ＰＣＲ法を用いたＤＮＡの増幅工程の前後の工程との継続性にも考慮した、デオキシリボ核酸の増幅装置及び増幅方法を提供することにある。

【０００５】

【課題を解決するための手段】上記目的は、反応液の気液界面からの水分蒸発が細管内の反応液をガスで封止することで実質的に防止できることに着目して、反応液を毛細管等の細管の中に入れ、前記した反応液を空気もしくは他のガスで両端を挟みこんだ状態にしてＰＣＲ法を行わせることにより達成される。

【０００６】

【作用】ＰＣＲ法を行うために反応液は、およそ９５°Ｃ程度の熱変性温度、およそ５５°Ｃ程度のアニーリング温度、およそ７０°Ｃ程度の重合温度の順に、通常数十回繰返し温度変化させられ、その際、水分蒸発が起きると反応液の組成が変化しPCR法が目的どおりに行えない。しかしながら、反応液からの水分蒸発は反応液とガスとの界面で起きるので、前記界面の面積を十分小さくすれば水分蒸発を十分小さくでき、ＰＣＲ法を行う場合に支障を生じない程度の反応液の組成変化にすることができる。更に前記ガスで反応液の移送を制御できるから、ＰＣＲ法を用いたＤＮＡ増幅工程の前後の工程との継続性にも考慮した、デオキシリボ核酸の増幅装置、増

幅方法が容易に実現できる。

【０００７】

【実施例】図１は一実施例を示す構成図で、１は内径が約１mmの毛細管、２は反応液供給口、３はガス給気口、４は反応液排出口、６は三方弁、７は止め弁である。８は毛細管支持具、９は毛細管移動機構であり、これにより毛細管支持具８の位置を制御する。両者の詳細は省略するが、要はスムーズに移動が制御できれば任意の構成が取り得る。２１ａ，２２ａ，２３ａは、それぞれ容器である。２１，２２，２３は熱媒体で、それぞれ容器２１ａ，２２ａ，２３ａに入っている。それぞれの熱媒体は、反応液の変性温度，アニーリング温度，重合温度に維持されている。１１は反応液であり、毛細管内にガスにより封止されている状態である。以下、図１に従って動作を説明する。予め毛細管１内や三方弁６止め弁７などの反応液の通過する部分を反応液の代わりに洗浄液を流すことにより反応液の汚染を防止し、毛細管移動機構９により毛細管支持具８によって毛細管１を熱媒体２１の中に入れたのち、三方弁６，止め弁７を操作し、それぞれ反応液供給口２と毛細管１，毛細管１と反応液排出口４とを連通させる。反応液１１を反応液供給口２より毛細管１の中に入れたのち、三方弁６を操作しガス給気口３と毛細管１とを連通させ、反応液１１が毛細管１内の所定の位置に来るようにガス給気口３よりガスを供給する。所定の時間後反応液１１が熱変性温度になったら、毛細管支持具８に連結された毛細管移動機構９を動作させて毛細管１を熱媒体２２の中に入れる。所定の時間後反応液１１がアニーリング温度になったら、毛細管支持具８に連結された毛細管移動機構９を動作させて毛細管１を熱媒体２３の中に入れる。所定の時間後反応液１１が重合温度になったら、毛細管支持具８に連結された毛細管移動機構９を動作させて毛細管１を熱媒体２１の中に入れる。以下、毛細管１の移動をおなじ順序で繰り返し所定の回数だけ熱変性温度，アニーリング温度，重合温度の順に反応液を温度変化させてＰＣＲ法を実施したのち、ガス給気口３よりガスを供給して反応液１１を反応液排出口４より排出する。以上の動作のなかで、三方弁６を操作しガス給気口３と毛細管１とを連通させ、反応液１１が毛細管１内の所定の位置に来るようにガス給気口３よりガスを供給する際に、止め弁７を操作し毛細管１内に適当な内圧がかかるようにしてもよい。このようにすると仮りに反応液１１中に微量の空気等のガスが混入していても反応液の温度変化によるガスの膨張に伴う反応液の分断を防止できる効果がある。

【０００８】いま一例として、内径１mm，外径２mmのプラスチック製の毛細管を用い、反応液を毛細管に入れた状態で９５℃の熱変性温度から５５℃のアニーリング温度の温水中に毛細管を入れたときの反応液の温度変化を数値計算で求め、反応液の平均温度の時間変化としてしめすと図２のようになる。この図から反応液の温度が約

１５秒で９５℃の熱変性温度からほぼ５５℃のアニーリング温度になることが分かる。即ち、毛細管の移動時間を入れても熱変性温度，アニーリング温度，重合温度の一連の温度変化に要する時間は約１分程度でありそれを３０回程度繰り返しても約３０分でＰＣＲ法を実施できる。計算結果は示さないが、内外径をそれぞれ１／２にすれば約１５分でＰＣＲ法を実施できる。

【０００９】図３は他の実施例を示す構成図で、１は毛細管、２は反応液供給口、３，５はガス給排気口、４は反応液排出口、６１，６２は三方弁、２１，２２，２３は熱媒体、２１ａ，２２ａ，２３ａは容器で、これに入っている熱媒体はそれぞれ熱変性温度，アニーリング温度，重合温度に維持されている。１１は反応液である。図３の実施例は、図１のそれに比し毛細管１の移動に代え反応液１１自体を移動させることとしたものである。以下、図３に従って動作を説明する。予め毛細管１内や三方弁６１，６２などの反応液の通過する部分を反応液の代わりに洗浄液を流すことにより反応液の汚染を防止したのち、三方弁６１，６２を操作し、それぞれ反応液供給口２と毛細管１，毛細管１とガス給排気口５とを連通させる。反応液１１を反応液供給口２より毛細管１の中に入れたのち、三方弁６１を操作しガス給排気口３と毛細管１とを連通させ、反応液１１が熱媒体２１に浸っている毛細管１内の所定の位置に来るようにガス給排気口３よりガスを供給する。所定の時間後反応液１１が熱変性温度になったら、反応液１１が熱媒体２２に浸っている毛細管１内の所定の位置に来るようにガス給排気口３よりガスを供給する。所定の時間後反応液１１がアニーリング温度になったら、反応液１１が熱媒体２３に浸っている毛細管１内の所定の位置に来るようにガス給排気口３よりガスを供給する。所定の時間後反応液１１が重合温度になったら、反応液１１が熱媒体２１に浸っている毛細管１内の所定の位置に来るようにガス給排気口５よりガスを供給する。以下、反応液１１の毛細管１内での移動を繰り返し所定の回数だけ熱変性温度，アニーリング温度，重合温度の順に反応液を温度変化させてＰＣＲ法を実施したのち、三方弁６２を操作し毛細管１と反応液排出口４とを連通させ、ガス給排気口３よりガスを供給して反応液１１を反応液排出口４より排出する。勿論、反応液を繰返しのために逆送するときは短時間で戻るように制御することとして、この逆送による影響の無いようにすることはいうまでもない。また、ガス給排気口３，５よりガスを供給して反応液１１の毛細管１内での移動を繰り返す場合に毛細管１内に適当な内圧がかかるようにしてもよい。この効果は第１の実施例と同様である。本実施例で、第１の実施例と同一の寸法の毛細管を使用した場合、毛細管は既に目的の温度になっているので反応液の温度が目的の温度になるのに要する時間は第１の実施例より短いことが容易に類推される。また、本発明では、反応液の移動がガスの給排気によりおこなわれるため、可動部がほとんどなく、安価で信頼性の高い装置とすることができる効果がある。

【００１０】図４は更に他の実施例を示す構成図で、１は毛細管で螺旋状に巻かれている。２は反応液供給口、３はガス給気口、４は反応液排出口、６は三方弁である。

【００１１】３１，３２，３３はヒートブロックでそれぞれ熱変性温度，アニーリング温度，重合温度に維持されており、毛細管１は、これに熱的に十分接触した状態で螺旋状に巻かれている。１１は反応液である。毛細管１の螺旋巻数は熱変性温度，アニーリング温度，重合温度の順に温度変化を繰り返すＰＣＲ法の必要な回数以上にする。以下、図４に従って動作を説明する。予め毛細管１内や三方弁６などの反応液の通過する部分を反応液の代わりに洗浄液を流すことにより反応液の汚染を防止し、三方弁６を操作し、反応液供給口２と毛細管１とを連通させる。反応液１１を反応液供給口２より毛細管１の中に入れたのち、三方弁６を操作しガス給気口３と毛細管１とを連通させ、反応液１１がヒートブロック３１の所定の位置に来るようにガス給気口３よりガスを供給する。所定の時間後反応液１１が熱変性温度になったら、反応液１１がヒートブロック３２の所定の位置に来るようにガス給気口３よりガスを供給する。所定の時間後反応液１１がアニーリング温度になったら、反応液１１がヒートブロック３３の所定の位置に来るようにガス給気口３よりガスを供給する。以下、同様の操作を繰り返し所定の回数だけ熱変性温度，アニーリング温度，重合温度の順に反応液を温度変化させてＰＣＲ法を実施したのち、ガス給気口３よりガスの供給速度を前記した速度より速くして連続供給し、反応液１１を反応液排出口４より排出する。前記した動作のなかで反応液１１が移動中に目的の温度になるように給気口３からのガス供給速度を適当に制御すれば、ガスの供給を連続的におこなってもＰＣＲ法を実施できる。なお、反応液排出口４の前後の適当な位置に絞り等の流体抵抗素子を設け毛細管１内に適当な内圧がかかるようにしてもよい。この効果は第１の実施例と同様である。本実施例で、第１の実施例と同一の寸法の毛細管を使用した場合、毛細管は既に目的の温度になっているので反応液の温度が目的の温度になるのに要する時間は第１の実施例より短いことが容易に類推される。また、本実施例では、反応液が一方向に移動することによりＰＣＲ法が行われるためガスの給気制御が容易であるとともに、反応液を不適切な温度状態にある場所を逆送する必要が無いから制御を高精度にできる。更に可動部がほとんどなく、安価で信頼性の高い装置とすることができる効果がある。

【００１２】反応液の界面での蒸発量について概算してみると次のようである。

【００１３】前記の蓋付のプラスチック容器に反応液を入れたときの界面の面積はおよそ３５mm^2、一方、内径

9

１mmの毛細管では両端合わせて約１．６mm²であるから界面の面積は１／２０以下になる。必要に応じてさらに細い内径の毛細管の使用すれば、界面の面積をさらに小さくすることも容易である。界面の面積を小さくすれば、水分蒸発を十分小さくできることは以下の事実でも証明される。内径１mmのプラスチック製の毛細管を用い、前記毛細管の中に反応液を入れ、前記した反応液を空気で両端を挟みこんだ状態にして９５℃の恒温室に１０分入れておいても、水分蒸発量は０．１％ 程度であった。温度が低ければ水分蒸発量がさらに小さくなることは自明である。すなわち、内径１mm程度以下の毛細管を反応液の容器として用い、前記毛細管の中に反応液を入れ、前記した反応液を空気もしくは他のガスで両端を挟みこんだ状態にすれば、従来技術で使用している鉱物油を使用しなくてよく、また、アナリティカル・バイオケミストリ，１８６（１９９０）第３２８頁から３３１頁（Analytical Biochemistry　１８６(１９９０）ｐｐ３２８－３３１）に提示されているように毛細管の前後を封止しなくてもＰＣＲ法を行う場合に支障を生じない程度の反応液の組成変化で目的とするＰＣＲ法が行える。

10

【００１４】

【発明の効果】以上説明したように本発明によれば、前記従来技術の欠点である、鉱物油の除去工程あるいは、毛細管の両端を燃焼ガスで封止する操作、ならびに封止した毛細管から反応液を取り出す操作が不要で、かつ、短時間で処理できるＰＣＲ法が実現できるだけでなく、反応液の供給，排出がそれぞれＰＣＲ法の前処理工程，後処理工程と連続できるよう工夫されているので、ＰＣＲ法を用いたＤＮＡの増幅工程の前後の工程との継続性のあるデオキシリボ核酸の増幅装置が実現できる。

【図面の簡単な説明】

【図１】本発明の一実施例を示す構成図である。

【図２】反応液の平均温度変化の一例を示す図である。

【図３】本発明の他の実施例を示す構成図である。

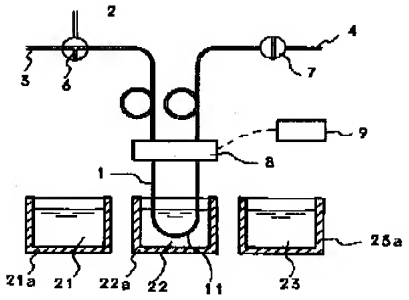
【図４】本発明の他の実施例を示す構成図である。

【符号の説明】

１…毛細管、２…反応液供給口、３…ガス給気口、４…反応液排出口、５…ガス給排気口、６，６１，６２…三方弁、７は止め弁、２１，２２，２３…熱媒体、３１，３２，３３…ヒートブロック

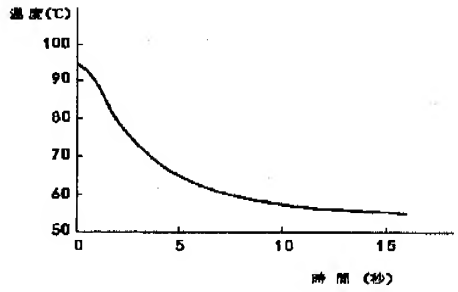
【図１】

図 1



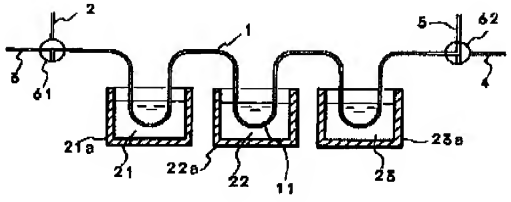
【図２】

図 2



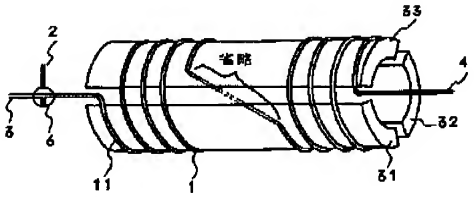
【図３】

図 3



【図４】

図 4



フロントページの続き

(58)調査した分野(Int.Cl.[7], ＤＢ名)
C12M 1/00
C12Q 1/68
ＢＩＯＳＩＳ（ＤＩＡＬＯＧ）
ＷＰＩ／Ｌ（ＱＵＥＳＴＥＬ）